Motion – Genetic testing is useful in the diagnosis of nonhereditary pancreatic conditions: Arguments for the motion

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Mutations of three major genes are associated with an increased risk of acute and chronic pancreatitis: the cationic trypsinogen (*PRSS1*) gene, the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, and the pancreatic secretory trypsin inhibitor (*PSTI*) or serine protease inhibitor, Kazal type 1 (*SPINK1*) gene. Some autosomal dominant forms of hereditary pancreatitis are associated with mutations of the *PRSS1* gene, which can be readily identified by genetic testing. Mutations of the *CFTR* gene can lead either to cystic fibrosis or to idiopathic chronic pancreatitis, and to a variety of cystic fibrosis-associated disorders, including congenital bilateral absence of the vas deferens and sinusitis. These mutations, as with those of the *SPINK1* (or *PSTI*) gene, are prevalent in North America; thus, the presence of such a mutation in an asymptomatic person does not confer a high risk of developing pancreatitis. Combinations of mutations of the *PRSS1* and *SPINK1* genes lead to more severe disease, as indicated by an earlier onset of symptoms, which suggests that *SPINK1* is a disease modifier. The major fear expressed by potential candidates for genetic testing is that the results could lead to insurance discrimination. Studies of the positive predictive value of genetic tests are hampered by recruitment bias and lack of knowledge of family history of pancreatitis. Genetic testing is most useful for persons for whom family members have already been found to exhibit a particular pancreatitis-associated mutation. In the future, increased knowledge of the myriad genetic causes of pancreatitis, as well as advances in the diagnosis and treatment of early chronic pancreatitis, should enhance the utility of genetic testing.

Key Words: *Cystic fibrosis transmembrane conductance regulator; Pancreatic secretory trypsin inhibitor; Serine protease inhibitor, Kazal type 1; Trypsin; Trypsinogen*

Motion - Les tests génétiques sont utiles dans le diagnostic des maladies pancréatiques non héréditaires : Arguments en faveur de la motion

RÉSUMÉ : Des mutations de trois gènes importants sont associées à un risque accru de pancréatite aiguë et chronique : le gène du trypsinogène cationique (PRSS1), le gène du régulateur de la perméabilité transmembranaire de la fibrose kystique (CFTR) et le gène de l'inhibiteur trypsique sécrétoire pancréatique (PSTI) ou de l'inhibiteur de protéase à sérine Kazal de type 1 (SPINK1). Certaines formes autosomiques dominantes de pancréatite familiale sont associées à des mutations du gène PRSS1 facilement décelables par test génétique. Les mutations du gène CFTR peuvent entraîner la fibrose kystique ou une pancréatite chronique idiopathique ainsi que divers troubles liés à la fibrose kystique, y compris l'absence bilatérale congénitale de canaux déférents et la sinusite. Ces mutations, à l'instar de celles du gène SPINK1 (ou PSTI), sont courantes en Amérique du Nord; par conséquent, leur présence chez une personne asymptomatique ne confère pas un risque élevé de pancréatite. L'association de mutations des gènes PRSS1 et SPINK1 entraîne des affections plus graves, comme en témoigne l'apparition plus précoce des symptômes, ce qui suggère que SPINK1 est un gène modificateur de la maladie. La principale crainte exprimée par les candidats éventuels à un test génétique est que les résultats pourraient mener à une discrimination de la part des compagnies d'assurance. Les études sur la valeur prédictive positive des tests génétiques sont entravées par des biais de recrutement et le manque de connaissances sur les antécédents familiaux de pancréatite. Les tests génétiques sont surtout utiles pour les personnes ayant de proches parents chez qui on a déjà mis en évidence une mutation associée à la pancréatite. Une meilleure connaissance des très nombreuses causes génétiques de la pancréatite et les progrès dans le diagnostic et le traitement précoces de la pancréatite chronique devraient permettre un jour d'accroître l'utilité des tests génétiques.

Some people see genetic testing as a Pandora's Box: "Don't open it!" Others see genetic testing as an incredibly powerful diagnostic tool that will accurately reflect the patient's past, present and future, and that of his or her family.

The issues surrounding genetic testing in gastrointestinal and liver diseases emerged in 1996 with the discovery of the common genetic defects for hereditary pancreatitis (1). By 1998, patients with idiopathic chronic pancreatitis were being screened for cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations (2,3). Finally, an association between idiopathic pancreatitis and mutations in the serine protease inhibitor, Kazal type 1 (*SPINK1*) gene was recognized in 2001 (4,5). Other pancreatitis mutations will soon be discovered. Genetic testing raises concerns among patients, gas-

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troenterologists, genetic counsellors, insurance companies, and lawyers. These concerns are real and will be addressed. In the future, however, genetic testing should be offered to all patients with suspected pancreatitis, especially early in the course of the disease. This may prevent delays in diagnosis and improve treatment options.

The focus of this article will be on the three primary genes that are known to be associated with pancreatitis, and the challenges and opportunities for the future.

THREE MAJOR PANCREATITIS-ASSOCIATED GENES

The primary genes that have thus far been shown to be associated with pancreatitis are the cationic trypsinogen gene (*PRSS1*), the *CFTR* gene, the pancreatic secretory trypsin inhibitor (*PSTI*), or (*SPINK1*), gene.

Each of these primary pancreatitis-associated genes teaches us something different about genetics and issues surrounding genetic testing.

PRSS1 MUTATIONS

Cationic trypsinogen is among the most abundant molecules produced by pancreatic acinar cells (6). It plays a central role in hydrolyzing dietary proteins at lysine and arginine amino acid residues, and also in activating all other digestive proenzymes (6). Premature activation of trypsinogen within the pancreas leads to pancreatic autodigestion and is believed to be a key step in the pathogenesis of acute pancreatitis. Recurrent attacks of acute pancreatitis, as occur in patients with hereditary pancreatitis, eventually lead to chronic pancreatitis.

Major mutations

Mutations in codons 29 (exon 2) and 122 (exon 3) of *PRSS1* cause autosomal dominant forms of hereditary pancreatitis (1,7-9). The codon 122 mutations usually result in an R122H substitution (older nomenclature, R117H [8,10,11]) that eliminates a 'fail safe' trypsin hydrolysis site in the side chain of trypsin that connects the two halves of the molecule. The elimination of this site causes a gain-of-function mutation because prematurely activated trypsin cannot be inactivated by autolysis (1,7,12). The N29I mutation (older nomenclature, N21I) causes a clinical syndrome identical to the R122H mutation syndrome, although the molecular mechanism causing the gain of function is still unclear (7,13). Other less frequent mutations at codons 29 and 122 have also been identified (14,15). The common N29I and R122H mutations occur in patients from North America (1,9), Europe (16-18), Japan (19), and probably elsewhere.

Clinical syndrome

Approximately 80% of persons with the *PRSS1* N29I or R122H mutations have recurrent episodes of acute pancreatitis during childhood. The median age of onset is roughly 10 years; chronic pancreatitis develops during the subsequent decade of the disease in about half of patients with acute pancreatitis, and pancreatic cancer occurs by age 70 years in 40% of individuals with chronic pancreatitis. Smoking more than doubles the risk of developing pancreatic cancer and reduces the median age of onset by 20 years (20).

Genetic testing

Genetic testing for hereditary pancreatitis has been available for research and clinical purposes for the past five years. The major issues surrounding genetic testing were recently reviewed (21-23). In general, the indications for clinical genetic testing vary widely according to the severity of the disease, the age of onset, the availability of surrogate markers and the possibility of an effective intervention. Patients are also likely to have their own reasons for pursuing genetic testing. Reasons for *PRSS1* mutation testing generally include verification of a clinical suspicion, helping patients to understand or validate their condition, and assisting individuals at risk of pancreatitis (and ultimately pancreatic cancer [24]) in making lifestyle decisions (involving diet and smoking, for example) that minimize the risk of disease (21). Indeed, identification of an established pancreatitis-associated gene mutation can be valuable in expediting the evaluation of recurrent pancreatitis in children and preventing the unnecessary pursuit of elusive causes of pancreatitis in adults (eg, sphincter of Oddi dysfunction or occult alcohol abuse). A consensus statement on genetic testing for suspected hereditary pancreatitis was developed at the Third International Symposium on Inherited Diseases of the Pancreas (in Milan, Italy, in April 2001), and has been published in a special issue of the journal *Pancreatology* (25). Such investigations are meant for patients with a clinical and family history suggestive of hereditary pancreatitis.

CFTR GENE

CFTR mutations causing cystic fibrosis

The primary *CFTR* mutation-related disorder is cystic fibrosis (CF), which is one of the most common life-threatening autosomal recessive disorders in Caucasians (26). Major mutations in both alleles of the gene result in the commonly recognized clinical features of CF: abnormal sweat chloride concentrations, neonatal hypertrypsinogenemia, pancreatic pseudocysts, fibrosis (hence the term 'cystic fibrosis'), chronic pancreatitis, and progressive pulmonary disease. Among CF patients, 66% have a three base pair deletion of the phenylalanine-coding codon 508 (F508), although approximately 1000 other mutations have been reported (27,28). African-Americans may have their own set of 'common' CF mutations, including the 3120+1G>A mutation, which occurs at a frequency of 12.3% in a representative population (29). Most *CFTR* mutations can be placed into one of five severity categories based on the observed or presumed molecular consequences (30,31). Typical CF patients with pancreatic insufficiency tend to have two severe mutations (ie, Class I, II, or III), whereas CF patients with pancreatic sufficiency from birth have at least one CF 'mild allele' (ie, Class IV or V) (31).

CFTR mutations associated with idiopathic chronic pancreatitis

In 1998, two groups reported a significant association between idiopathic chronic pancreatitis and various *CFTR* mutations

(2,3). Indeed, several mild, 'pancreas sufficient' mutations (eg, *CFTR* R117H and the intron 8 '5T allele' [IV8 5T], which results in an 80% reduction of exon 9 expression [32,33]) seem to be associated with idiopathic chronic pancreatitis (2,3) and other features of CF, including congenital bilateral absence of the vas deferens (32,34). Other mild *CFTR* mutations (eg, L997F [35]) may also be associated with neonatal hypertrypsinemia and/or idiopathic pancreatitis but not with lung disease or abnormal sweat chloride. Although initial reports suggested that idiopathic chronic pancreatitis was associated with a single allelic mutation of *CFTR*, more recent evidence suggests that patients with chronic pancreatitis may actually have compound heterozygous mutations of *CFTR* and mild CF. This argument is based on at least three observations:

- Healthy parents of children with CF (obligate *CFTR* mutation carriers without CF) do not have an increased incidence of acute or chronic pancreatitis compared with the general population (36).
- The prevalence of functional classes IV and V *CFTR* mutations (eg, R117H, IV8 5T) among patients with idiopathic chronic pancreatitis without a coexisting severe mutation is only about 1.5-fold higher than the expected number (which is not statistically significant) (37).
- Severe and mild *CFTR* compound heterozygous mutations are strongly associated with idiopathic chronic pancreatitis, and the majority of these patients have abnormal nasal bioelectric responses, thus demonstrating *CFTR* dysfunction (37).

Therefore, a subset of patients with chronic pancreatitis appear to have atypical CF. Indeed, genetic screening of infants who were thought to be heterozygous F508 carriers (on the basis of a normal sweat chloride but elevated immunoreactive trypsinogen, which is associated with pancreatic injury) frequently yielded evidence of R117H mutation (9%) or IV8 5T allele (20%) (38). These data led to the following conclusions (2,3,37):

- Individuals with a single mutant *CFTR* allele are asymptomatic carriers.
- Persons with two severely affected *CFTR* alleles have classic CF.
- Persons with compound heterozygous *CFTR* genotypes with a severe (classes I to III) and a mild (class IV or V) mutation may be at risk for pancreatitis, congenital bilateral absence of the vas deferens (39,40), sinusitis, or other CF-associated disorders.

Genetic testing

CFTR is a large molecule with 1480 amino acids coded for by over 4400 nucleotides in 24 exons (26). Idiopathic chronic pancreatitis appears to be associated with some loss of *CFTR* function, which can be caused by many combinations of *CFTR* mutations. However, screening of the entire *CFTR* gene for mutations is difficult and very expensive, thereby limiting this approach to specialized research laboratories. Some commercial laboratories do offer clinical testing for a panel of mutations that are commonly associated with CF, but these panels may not include many of the 'mild' *CFTR* mutations that are associated with pancreatitis (37). Furthermore, since *CFTR* mutations are prevalent in the population, the identification of a polymorphism does not necessarily mean that it is the cause of pancreatitis. Nor does the presence of a *CFTR* polymorphism confer a high risk of pancreatitis in asymptomatic individuals. For example, the incidence of acute pancreatitis in the general population is 1 in 16,000 (41), and the presence of the *CFTR* R117H genotype increases the risk 2.6-fold (37); thus, the overall risk of pancreatitis with this mutation is only 2.6 in 16,000, or 0.16%.

A major dilemma regarding genetic testing of patients with recurrent acute or chronic pancreatitis has already arisen. Cohn et al (37) suggest that, at the present time, *CFTR* testing may be considered for individuals in whom pancreatitis appears to be the earliest manifestation of classic CF, or for young patients with pancreatitis and borderline sweat chloride values, so that individuals can be referred to CF centres or for genetic counselling. The situation is becoming more complicated, however, because the World Health Organization (WHO) is about to publish the Classification of Cystic Fibrosis and Related Disorders in the upcoming International Classification of Diseases (ICD-11). Dodge reported on the deliberations of several major European working groups during the Third International Symposium on Inherited Diseases of the Pancreas in Milan, Italy, on March 4 to 6, 2001; the conference proceedings have also been published (42). According to these guidelines, all patients with 'chronic pancreatitis' require testing for *CFTR* mutations, and the presence of even a single *CFTR* mutation identifies the patient as having a CF-associated disorder. This formulation raises a number of serious questions for which there are no answers, including: Which mutations must be considered? How can a single common *CFTR* mutation be diagnostic of CF-associated chronic pancreatitis? Is testing for *CFTR* mutations always warranted in patients with chronic pancreatitis?

The author believes that *CFTR* mutation analysis may play an important role in the future, but most of the work has been on severe CF-causing mutations in Caucasians, and large national studies have not been conducted for other ethnic populations.

SPINK1/PSTI GENE MUTATIONS

SPINK1 function

PSTI, which is also known by its UniGene name, *SPINK1*, is a 56-amino acid peptide that specifically inhibits trypsin by physically blocking the active site. *SPINK1* is synthesized by pancreatic acinar cells together with trypsinogen and is located in the same zymogen granules. In the mechanistic models of pancreatic acinar cell protection, *SPINK1* acts as the first line of defence against prematurely activated trypsinogen

(1,4-6,43). Because only a limited amount of *SPINK1* is produced, however, it is capable of inhibiting only approximately 20% of the trypsin that could potentially be formed.

SPINK1 mutations

Because gain-of-function mutations of the trypsin molecule are associated with acute and chronic pancreatitis, it was hypothesized that loss of trypsin inhibitor capacity might have similar effects. By 2000, it was appreciated that *SPINK1* mutations indeed predispose to chronic pancreatitis (4,5,44). This extremely important discovery was made by screening a large, well-defined population for mutations in candidate genes. *SPINK1* N34S and P55S mutations are relatively prevalent, being present in roughly 2% of the general population (5,44). In addition, a number of intron mutations, which have not been fully characterized, occur in patients with pancreatitis (unpublished observations). *SPINK1* mutations occur in families of pancreatitis patients who do not have trypsinogen mutations, but the mutations do not consistently segregate with the disease (5,44). Thus, *SPINK1* mutations do not cause hereditary pancreatitis in an autosomal dominant pattern. Proof of the role of *SPINK1* mutations, however, is the finding that 23% to 25% of patients with idiopathic chronic pancreatitis harbour them (4,5).

Gene-gene interactions

We have argued that *SPINK1* mutations act as disease promoters in that they lower the threshold for initiating pancreatitis or possibly worsen the severity of pancreatitis that is caused by other genetic or environmental factors (5). It has also been shown that the presence of *PRSS1* and *SPINK1* mutations in the same person influences the phenotype (45). The author's group and the Midwest Multicenter Pancreatic Study Group have collected a large database from kindreds of patients with hereditary pancreatitis; it currently includes 717 individuals, of whom 368 (51%) have confirmed pancreatitis. Unaffected individuals include relatives and spouses, who are essential for conducting genetic linkage studies, as well as 46 relatives who were found to be silent carriers for a *PRSS1* mutation. From this data set, 39 persons were identified with *PRSS1* mutations but without *SPINK1* mutations, and 22 with both *PRSS1* and *SPINK1* mutations. The median age of disease onset was older for patients with mutations of *PRSS1* alone than for patients with mutations in both genes. The possible interaction between *SPINK1* mutations and *CFTR* mutations may also be important. Because the *SPINK1* mutation is not sufficient to cause disease in most individuals (as already mentioned), the significantly earlier age of onset seen in persons with both *PRSS1* and *SPINK1* mutations supports the concept that *SPINK1* acts as a disease modifier.

Genetic testing for *SPINK1* mutations

The discovery of this disease-associated mutation raises the question of when to undertake genetic testing. Identification of *SPINK1* mutations in patients with early chronic pancreatitis may provide important information about the cause of the disorder. On the other hand, because fewer than 1% of persons with heterozygous *SPINK1* mutation alone are at risk of developing pancreatitis, it is not worthwhile to test asymptomatic subjects. *SPINK1* mutations are associated with early onset of symptoms; therefore, testing is unlikely to be fruitful in patients who develop pancreatitis after the age of 20 years.

CONCERNS AND OPPORTUNITIES

Fears about genetic testing

The decision about whether to proceed with genetic testing is often complex. Currently, the utility of this technique is limited by our incomplete understanding of the etiology of pancreatic disease and our inability to use genetic information to alter the clinical outcomes. Even though these factors will change in the future, the patients' fears of genetic testing result in significant hesitation.

The author's group recently studied the motivations and concerns of patients who were offered genetic testing for hereditary pancreatitis (22). By far, the major concern was insurance discrimination; this is the case even with hemochromatosis, a disease in which genetic testing can identify patients who could be effectively treated with phlebotomy. In a recent study, more than 25% of 124 asymptomatic subjects who were identified as hereditary hemochromatosis carriers reported that they were either required to pay higher premiums or denied coverage altogether (46).

Unfortunately, as of 2001, fewer than half of the states in the United States (Arizona, California, Colorado, Connecticut, Florida, Georgia, Hawaii, Illinois, Maryland, Minnesota, Nevada, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oregon, Tennessee, Texas, Virginia and Wisconsin) have passed legislation prohibiting genetic discrimination in health insurance (46). Participation in a research study, rather than attendance at a clinical laboratory, is an attractive option for individuals who do not want their test results to be included in their medical records. In this setting, the results are disclosed directly to the patient, who can then decide to whom the results will be disclosed.

Accuracy of testing

Modern and properly applied genetic testing techniques yield positive and negative predictive values of almost 100% for identifying specific mutations. The pretest probability of a test for a *PRSS1* mutation depends on several factors. The strongest predictors of a genetic basis for a case of pancreatitis include a typical family history (especially an autosomal dominant inheritance pattern) and an early age of onset of symptoms.

A delayed onset of pancreatitis (ie, over age 20 years) does not preclude a genetic etiology. Although 93% of patients in the Midwest Multicenter Pancreatic Study Group-Pittsburgh study developed symptoms by the age of 30 years (45), only 40% of affected persons enrolled in the European Genetic Register of Hereditary Pancreatitis and Familial Pancreatic Cancer study became ill by that age (21). These conflicting results suggest the possibility of selection or recruitment bias in studies that were not designed to explore the age of onset of symptoms. The latter study (21) might have overestimated the age of onset of disease because of a failure to fully elucidate the patients' histories.

Impediments to investigation include the facts that many patients are unaware of their family histories, have few relatives, have unaffected first-degree relatives or have ancestors in whom the diagnosis of pancreatitis was not established (unpublished observations). Moreover, the underlying frequency of mutations varies across populations. For example, the prevalence of *PRSS1* mutations ranges from 0% to 19% among various patient populations with presumed idiopathic chronic pancreatitis (18,47-49), perhaps because of differences in regional settlement patterns. The likelihood of positive test results is much higher in endemic regions.

Interpretation of test results

The results of genetic testing have implications for both the subject and his or her extended family. Roughly 80% of individuals with either the R122H or the N29I trypsinogen mutation will experience at least one episode of acute pancreatitis (ie, the disease penetrance is 80%) (50-53). About one-half of clinically affected persons with either mutation will develop symptomatic chronic pancreatitis, and there is an increased risk of pancreatic cancer (23,54). Furthermore, there is a 50% chance of passing on the mutation to each child.

In asymptomatic individuals, a positive test result signifies an increased risk of pancreatitis, which possibly diminishes with age. A negative test result in a patient whose family is known to harbour a pancreatitis-associated gene mutation essentially eliminates the risk of developing that particular genetic form of pancreatitis. On the other hand, if such a mutation has not been previously identified in the family, then a negative test result in an asymptomatic person is considered 'noninformative' because the subject might have inherited a different pancreatitis-causing mutation.

The situation with *CFTR* and *SPINK1* mutations is more complex because pancreatitis-associated mutations occur in more than 1% of North American Caucasians, even though the prevalence of chronic pancreatitis is small (1 of 16,000 persons). Identification of one of the common pancreatitis-associated mutations of these genes in a patient with pancreatitis suggests that the mutation contributed to the etiology of pancreatitis, whereas the same mutation in an unaffected individual confers only a slight risk (approximately 5%) of ever experiencing pancreatitis.

Future developments

The major questions regarding genetic testing in the future will be who to test and when to test them. The technique is appropriate when the following conditions are met:

1. The patient will derive clinical benefit from the test results.
2. The test addresses the clinically relevant mutations.
3. The test is affordable, reliable, and easy to perform.
4. Insurance discrimination and other disincentives are minimal.
5. The patient can give informed consent.
6. The results are properly interpreted and communicated.

It is clear that testing should be undertaken for markers of a very high risk for pancreatitis, such as the *PRSS1* R122H and N21I mutations (21-23).

Greater opportunities for genetic testing will come with better understanding of the genetic and environmental risk factors for pancreatitis. This goal can be accomplished by means of national studies involving thousands of patients and appropriate controls. We must also develop sensitive and specific methods for the early detection of chronic pancreatitis, and find ways to limit disease progression.

The development of new molecular diagnostic methods that allow for the simultaneous screening of hundreds of disease-associated mutations would make genetic testing extremely useful. Once these conditions are met, patients with typical features of pancreatitis should be surveyed for environmental factors and offered genetic screening to determine their risk for pancreatitis. Patients who are shown to be at increased risk would then undergo a sensitive and specific confirmatory test (for example, endoscopic ultrasound with biopsy [23]). Effective therapy could be initiated for patients with the diagnosis of early pancreatitis. In this way, a disease like chronic pancreatitis, which cannot be cured, can be readily prevented.

Indeed, we must risk opening the mystical box; is it Pandora's, or panacea's, or somewhere in between?

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